ISCHEMIA

Succinate comes up ROSes

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NATURE

Ischemia is caused by a lack of oxygen due to the blockage of blood flow during a heart attack or stroke. Subsequent restoration of blood flow (reperfusion) is associated with the accumulation of mitochondrial reactive oxygen species (ROS), which cause oxidative damage. Although the role of ROS in ischemic damage has been validated, it was not known what factors mediate the elevation in ROS production and whether this is a direct or indirect response to ischemic conditions. Chouchani *et al.* hypothesized that a metabolic signal might stimulate ROS formation. To test this hypothesis, they first performed LC/MS-based metabolomics analysis of four tissues (kidney, liver, heart and brain) under ischemic conditions. The authors found that the TCA cycle intermediate succinate was consistently elevated in all examined tissues during ischemia and was rapidly oxidized during reperfusion. Although succinate is generated by the citric acid cycle using either glucose, fatty acids or glutamate or by the GABA shunt, none of these sources contributed to the elevated succinate levels. Instead, *in silico* flux analysis suggested that the source of the increased succinate was from the reversed activity of succinate dehydrogenase (SDH), reducing fumarate to succinate. The excess fumarate was thought to derive from two major pathways: the malate/aspartate shuttle and the purine nucleotide cycle. Additional flux analysis predicted that succinate is oxidized during reperfusion and promotes superoxide formation through mitochondrial complex I–mediated reverse electron transport (RET). Using a cell-permeable derivative of succinate and an inhibitor of complex I RET, the authors demonstrated that high levels of succinate along with a strong proton motive force were sufficient to induce ROS formation. Finally, the addition of dimethylmalonate, a cell-permeable precursor of the SDH competitive inhibitor malonate, reduced succinate and mitochondrial ROS levels during ischemia and exhibited protective effects in a cardiac and brain ischemia model. These findings may inspire new ways to modulate succinate metabolism for potential treatments of cellular damage caused by ischemia-reperfusion injury. *GM*

NITROGEN METABOLISM

Plants pocket glutamine

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The amino acid glutamine is a hub for nitrogen metabolism, accepting reduced nitrogen in nitrogen assimilation pathways and acting as an amino group donor throughout central and secondary metabolism. P_{II} signaling proteins, which are found in bacteria, plants and archaea, are conserved sensors of cellular nitrogen metabolism. Earlier studies have shown that P_{II} proteins in the chloroplasts of plants and cyanobacteria activate *N*-acetyl-L-glutamate kinase (NAGK)—a gateway enzyme in the arginine biosynthesis pathway—by formation of a specific P_{II}–NAGK complex. Unlike in prokaryotes, P_{II} proteins in plants have a C-terminal extension that has an unknown function despite being highly conserved. Biochemical and structural studies by Chellamuthu *et al.* now show that this newly termed 'Q-loop' creates a glutamine binding pocket on P_{II} proteins that couples glutamine sensing directly to NAGK regulation. Recombinant P_{II} proteins from the green alga *Chlamydomonas reinhardtii* and from *Arabidopsis thaliana*, which has a small deletion in the C-terminal extension, have almost identical biochemical properties. However, enzyme assays and surface plasmon resonance experiments on the *C. reinhardtii* system revealed that free glutamine and the C-terminal Q-loop of *C. reinhardtii* P_{II} (CrP_{II}) are required for it to bind and efficiently activate *Cr*NAGK. To gain more molecular insight, the authors solved the X-ray crystal structure of a CrP_{II}–*At*NAGK complex and found it to be virtually identical to the known complex of *Arabidopsis* proteins, featuring a hexameric toroid of NAGK capped on both sides by P_{II} trimers. However, differing from the *Arabidopsis* case, the Q-loop of CrP_{II} is organized into a helix-turn-helix motif that specifically binds glutamine and positions the T-loop of CrP_{II}, the P_{II} domain responsible for NAGK activation, for efficient engagement with NAGK. Comparative sequence analysis and characterization of the biochemical properties of P_{II} proteins from rice and a moss demonstrated that Q-loop–dependent glutamine sensing occurs in all plants except those in the *Brassicaceae* family, to which *A. thaliana* belongs. *TLS*

METALS

Regulate good times, c'mon!

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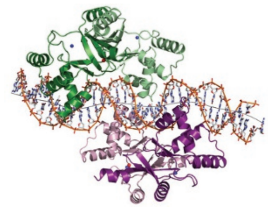
PLoS BIOLOGY



Bacteria have several families of metal-binding proteins that enable them to survive when the extracellular concentrations of metal ions are very low or extremely high. One such protein—the zinc uptake regulator (Zur) protein—is a transcription factor that maintains zinc homeostasis in *Escherichia coli* by regulating the expression of several proteins, including zinc transporter proteins, a ribosomal protein and a periplasmic zinc-trafficking protein. Gilston *et al.* have now solved the X-ray crystal structure of two Zur dimers bound to a DNA duplex derived from the *znuABC* operator ($(Zur_2)_2$–P_{znuABC}). The authors identified two zinc-binding sites in each of the Zur monomers and determined that mutations that eliminated metal binding abolished Zur-mediated transcription *in vivo*. Native gel shift experiments were used to show that the binding of the two Zur dimers to the *znuABC* promoter was highly cooperative, and the authors determined that a pair of salt bridges that link the Zur dimers mediates the observed cooperativity. Examination of the protein–DNA interface in the $(Zur_2)_2$–P_{znuABC} structure and the sequences of two other Zur-binding promoters yielded a putative Zur recognition sequence. This DNA sequence was used to identify a previously unknown Zur-binding promoter in *E. coli*, upstream of the periplasmic lysozyme inhibitor *pliG* gene. Comparison of the binding affinities for this transcription factor and the four known Zur-binding promoters indicated that there is a ~20,000-fold difference in affinity between the strongest and weakest Zur–DNA interaction. These *in vitro* binding affinities nicely correlate with the levels of repression observed *in vivo*, as the promoters with tightest Zur binding interactions are the most strongly repressed by Zur in cells (and vice versa). Additional work is needed to determine whether the *in vitro* thermodynamic properties of other metal- and DNA-binding proteins correlate with their behavior *in vivo*. *JMF*

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